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Behaviors of Rats With Insidious, Multifocal Brain Damage Induced by Seizures Following Single Peripheral Injections of Lithium and Pilocarpine

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PERSINGER, M. A., Y. R. J. BUREAU, M. KOSTAKOS, O. PEREDERY AND H. FALTER, H. *Behaviors of rats with insidious, multifocal brain damage induced by seizures following a single peripheral injection of lithium and pilocarpine.* PHYSIOL BEHAV **53**(5) 849–866, 1993.—Several domains of behavior were measured in rats (n = 465) 10 days to 100 days after induction of limbic seizures by a single subcutaneous injection of lithium and pilocarpine. These rats displayed enhanced intragroup aggression but normal muricide; gustatory neophobia and conditioned taste aversion were virtually eliminated. Severe working and reference memory deficits were evident within the radial arm maze. Both state-dependent memory and possible situation-dependent precipitation of spontaneous seizures were suggested. The behavioral changes were considered commensurate with the multifocal pattern of thalamic, hippocampal/amygdaloid, and limbic cortical damage.

Radial arm maze (RAM) Conditioned taste aversion (CTA) Lithium Limbic seizure Memory Acetylcholine Brain damage

THE traditional approach to understanding the relationship between brain, physiology, and behavior usually involves the disruption of singular regions by experimental lesions. We assume that the brain is an aggregate of functional systems. Each system is composed of a matrix of neuronal groups that can contribute differentially to various domains of behavior. In order to examine these systems or matrices as functional units, the entire aggregate must be affected. When these systems are eliminated, entire domains of behavior, e.g., maternal behavior, can be abolished (30).

Systemic induction of electrical seizures is one method by which the experimenter can affect a matrix of brain elements that are structurally and functionally associated. If the amount of correlative cell necrosis can be controlled, then one can trace (histologically) the pathways of the electrical seizures and quantitatively determine the relative contribution of each element (nuclear group) to the matrix. The relative weighting of each element to the matrix can be inferred by the proportional loss of cells.

Seizure-induced multifocal necrosis within functionally related systems within the diencephalon and telencephalon of the rat brain was first observed by Honchar, Olney, and Sherman (15). They reported that limbic electrical seizures and forelimb clonus in the rat could be precipitated by one-tenth of the normal subcutaneous dosage of pilocarpine if the rat's serum contained (human) therapeutic levels of lithium. Unfortunately, the subsequent status epilepticus resulted in extremely high mortality (46) rates (usually within 24 h) and, hence, precluded the pursuit of any behavioral correlates. We have found that a single SC injection of acepromazine minimizes this mortality.

This paper reports the first phase of relating different domains of behavior to multifocal lesions within functionally related brain structures. The effects of seizure-induced cell death upon two types of aggressive behavior and two major classes of learning and memory—radial arm maze (RAM) and conditioned taste aversion (CTA)—were examined. Possible incidental learning (or conditioning) of spontaneous seizures was also determined.

A multifocal (matrix) approach to brain function is considered ecologically relevant in light of the typical clinical manifestations of traumatic or idiopathic brain injury. Closed head injuries (CHI) usually involve more than one cortical or subcortical region due to either direct or subsequent transneuronal degener-

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ation. Complex partial epilepsy (37) and even normal geriatric progression (43) recruit distal and diverse brain structures that are related to the same functional systems.

LONG TERM CHARACTERISTICS AND CONSEQUENCES OF SEIZURES: A GENERAL SUMMARY

The characteristics of the initial seizure that is induced by the single injection of either 2 mEq/kg or 3 mEq/kg injection of lithium followed 4 h or 24 h later by 20 mg/kg or 30 mg/kg of pilocarpine have been described in our previous paper (34). Seizure onset times (SOTs) for male adult rats range between 20 and 40 min. The relationship between the lithium dosage and the SOT is relatively linear, and the method of ingestion does not alter the SOTs. Figure 1 shows the correlation between SOT and the amount of lithium (masked in 10% sucrose) that was consumed 24 h before a subcutaneous injection of 30 mg/kg of pilocarpine.

Reversal of the order of the injections has never evoked any seizures. For example, none of the 10, 150-day-old Wistar females injected with 30 mg/kg of pilocarpine and then 4 h later with 3 mEq/kg of lithium displayed overt seizures. All of the 10 females of the same age injected with the same dosages in the normal temporal order displayed the classic seizure pattern.

In order to facilitate survival, rats are injected Sc with 25 mg/kg of acepromazine (Ayerst Laboratories, Montreal) within 30 min of the onset of the seizure. Without this injection, mortality during the first 48 h after the induction of the seizure is about 95% for males (n = 200) and about 60% for females (n = 150). Histopathological analyses indicate that the duration between the seizure onset time and the injection of the acepromazine is directly proportional to the severity of the necrosis; it develops primarily within thalamic and entorhinal regions during the subsequent weeks.

Major behavioral changes occur during the weeks that follow the induction of the seizure. During the first 24 h after the seizure onset, most animals remain comatose; those who display frank whole body hypotonicity and hypothermia usually never recover from coma and die within 24 h to 48 h. Histological analyses of their brains indicate pervasive transcortical cell loss and necrosis, particularly within the outer three layers.

For those animals that are greater than 100 days of age at the time of seizure induction (by 3 mEq/kg of lithium followed 24 h later by 30 mg/kg of pilocarpine), relative weight losses of 15% to 20% occur within 48 h. The loss cannot be attributed totally to the absence of food or water intake because control rats deprived of food and water loose between 5% and 10% of their weight. During the first 5 days following seizure induction, most rats respond to a food mush (chow dust in water); they avoid licking contact with water and employ whole-mouth consumption of food or water. Sudden death occurs in about 10% of the male rats, even if they were administered the postseizure acepromazine.

Seven to 10 days after the seizure induction most rats begin to eat dry food chow, if available on the cage floor, and to drink from the water bottle. About this time rats that are housed in groups begin to display frequent (loud) teeth grinding and gnawing. Tails of cage mates are speckled with dozens of pin-point incisions. When placed in open fields that contain food, these rats immediately engage in gnawing, a behavior that is never seen in ad lib controls. The gnawing can be blocked transiently by 0.4 mg/kg of Haldol, the same dosage that blocks tail pinch-induced gnawing (4). At the same time, intragroup aggression escalates significantly. About 20% of the rats housed in pairs display synchronous stereotyped movements with their partners; the durations of the episodes range between 10 min and 15 min.

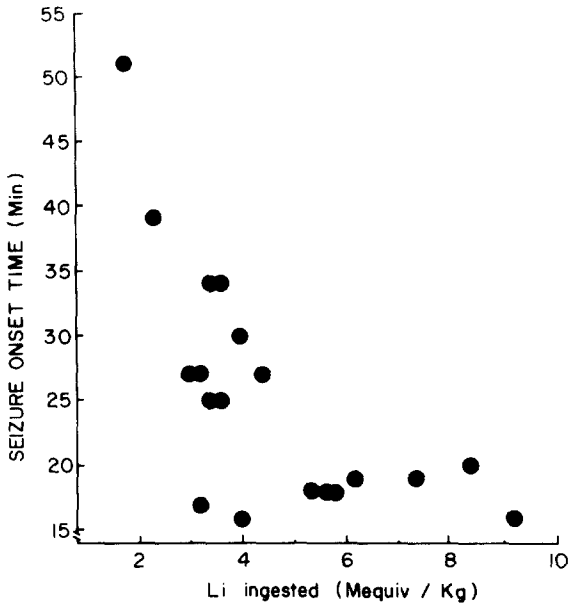

FIG. 1. Seizure onset time (SOT) as a function of the lithium ingested (mEq/kg) in a 10% sucrose solution; it was consumed 24 h before the subcutaneous injection of 30 mg/kg of pilocarpine.

In about 20% of the animals, the aggression is extreme. If they are not separated for humane reasons, ears and tails are removed and substantial blood loss occurs.

Between 10 days and 300 days (longest observation) after the seizure induction, rats will intermittently display episodes of staring that are followed within 10 s to 15 s by forepaw clonus and rearing. They are stereotyped and always display one of two outcomes. The first form involves an apparent return to a normal state after the rearing and forepaw clonus. However after a latency of about 30 s, the rat responds explosively to sound, touch, or vibrissae (weak air pressure) stimulation, by rapid thrashing and escape sequences; most researchers would label this behavior as an intense fear response. The second form involves immediate generalization into a clonic–tonic convulsion; when this occurs, fear responses are less frequent.

Except for the gnawing and occasional overt seizure, the rats' gross behaviors appear to be relatively normal. However, the skilled observers and technicians who have handled the rats report some distinct features. Seizured rats display an exaggerated ear twitch and persistent partial rotation of the pinnae, increased activity when placed in open areas, hyperreactivity to handling, and a propensity to fall from edges of transport cages. Rats that loose weight, despite attempts to correct this deficit, display near-continuous ambulation within the home cage. Sperm plugs are frequently evident below the cages.

Body weights for most of these rats ultimately return to the preseizure values, but subsequent weight gain does not occur. In one pilot study the weight/initial weight ratios for 12 rats at 30, 60, and 90 days after they had received the lithium/4-h/pilocarpine treatment were 0.92, 0.96, and 1.01, respectively. For the same period, mean values for four control rats were 1.14, 1.23, and 1.33. There were no qualitatively distinctive signs that the animals were consuming less food or water.

An increased mortality in rats that have been seizured has also been clearly evident in our studies. If rats survive the first 5 days after seizure induction, then the likelihood is about 98% that they will survive for at least 30 days. Between this time and

90 days after the seizure, survival percentages drop to about 63%. About 80% of these cases were sudden death, while another 20% were associated with slow attrition that was indiscriminable from the behaviors evoked by the initial seizure induction. For reasons that are not clear, the mortality is negligible for rats that are involved in behavioral experiments.

Several attempts have been made to quantify the daily incidence of seizures during the months that follow the lithium-pilocarpine injections. Continuous monitoring by jiggle cage or infrared photocell counts for activity patterns is not sensitive to the brief seizure. Sampling procedures, based upon entering the housing area randomly 10 times per day for 5 min during the day cycle, and continuous observation during the 1 h before and 1 h after the onset of the daily light cycle indicates an average overt seizure display of about 0.5 to 1 per day per rat. Indirect evidence that the seizures continue subclinically or that some insidious process is operative can be seen in Fig. 2. A positive correlation exists between the postseizure time and the relative enlargement of the lateral ventricles (ventricle area divided by the area of coronal section) at the level of the septum.

During the last 3 years, the neuroscience group has been evaluating the specific pattern of damage microscopically at 100 × to 400 ×. Although a scaling system has been devised that reflects progressive necrosis, the simple designation of the presence of damage (which is always visually conspicuous) or absence of damage within a nucleus is informative. Table 1 indicates the percentage of rats (31 successive brains) that displayed damage in nuclei or structures within the mesencephalon, diencephalon, and telencephalon (we have not investigated the submidbrain structures, histologically). All structures were differentiated according to the designation of Paxinos and Watson (29). Conservatively, using Friedman's nonparametric (repeated measure) criterion, the percentage of damage within any two structures for this sample must differ by about 25% to be statistically significant at the $p < 0.05$ level.

Seizures had been induced in each of these rats by 3 mEq/kg of lithium followed either 4 h or 24 h later by 30 mg/kg of pilocarpine; brains were fixed and processed between 10 and 30 days after the seizure onset. The most frequently and severely damaged structures are the entorhinal and piriform cortices, the substantia nigra (reticulata), the dorsomedial thalamic nuclear group, the posterior thalamic group, and (most of) the amygdaloid region. During this period some neuronal dropout in the neocortices was also evident.

There are three types of lesions that are relatively specific to particular areas. Within the thalamus the seizure-induced damage is manifested by neuronal dropout and necrosis (Fig. 3). Lesions within the entorhinal cortices are dominated by massive cellular loss and cystic formations (Fig. 4). Specific lines of dense gliosis (Fig. 5) occur within the pars reticulata of the substantia nigra, a change that has been used to explain the release of incessant gnawing by these animals.

Within susceptible regions, damage is usually evident within 48 h after the induction of the seizure. About 50 days after the seizure induction, the diffuse Nissl staining material that had been evident since about postseizure day 10 within the thalamus only becomes aggregated into amorphous deposits with diameters between 500 μm and 10,000 μm; by postseizure day 70, this material appears crystalline in some rats (Fig. 7). We have associated the consistent occurrence of this material within the supragenicular and adjacent medial geniculate with the lowered threshold and increased amplitude of movement and orientation of the ears, especially towards emotionally significant stimuli (unpublished data); this supposition has been supported by the

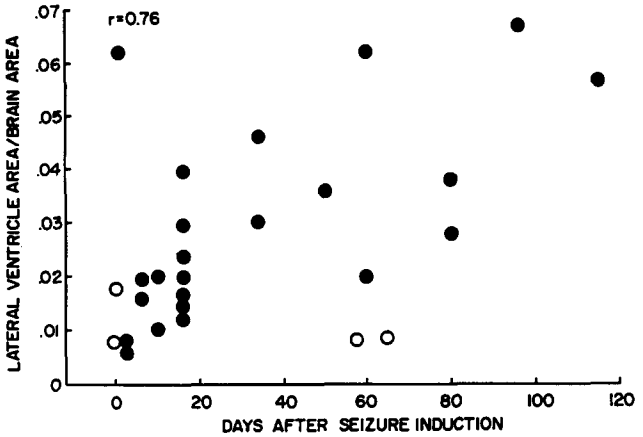

FIG. 2. Ratio of the cross-sectional area of the lateral ventricles at the level of the septum compared to the area of the coronal section as a function of time since the induction of the seizure (closed circles). Open circles indicate brains of control rats.

by the results of posterior thalamic-amygdaloid mapping through retrograde transport methods (24).

Location of this crystalline material is primarily within those nuclei that displayed the most severe neuronal death. Histochemical analyses indicate intense calcium staining within these aggregates, a finding that has been verified by atomic absorption (AA) analyses (20); these measures revealed no anomalous concentrations of either lithium, magnesium, or zinc. The calcium is embedded within a neutral mucopolysaccharide complex that contains either a nucleic acid or protein moiety; our attempts to verify the presence of an amyloid variant have not been successful.

METHOD

Subjects

A total of 465 Wistar albino rats, obtained from Charles River Breeding facilities, served as subjects. Unless otherwise specified, rats were male, between 90 and 120 days of age at the time of seizure induction or selection as controls, and had been housed in groups of three within temperature-regulated (22°C) rooms. The LD cycle was 12:12; light onset was 0700 LT.

Procedure

A total of five major experiments were completed over a 2-year period. Because each contained subsets of experiments, the procedures will be described separately. Unless stated specifically, all rats received SC injections of the drugs and had received 25 mg/kg of acepromazine within 1 h of the pilocarpine injection (about +30 min after seizure onset). All analyses involved SPSSx software on a VAX 4000 computer.

Experiment I: Sources of mortality and measures of recovery. In part A, 46 rats were given 3 mEq/kg of lithium followed 24 h later by 30 mg/kg of pilocarpine. One hour after the seizure onset, they were given either 10 mg/kg or 25 mg/kg of either acepromazine (Atravet) or chlorpromazine, i.e., Largactil (n = 10/group). Because previous data (34) clearly indicate that rats die without acepromazine treatment, only six rats were selected as controls.

In order to insure (part B) that the apparent recovery from the status epilepticus (following the acepromazine) was not associated with covert distress, 58 6-month-old female rats were

TABLE 1

PERCENTAGE OF RATS IN WHICH LIMBIC SEIZURES HAD BEEN INDUCED BY SUBCUTANEOUS INJECTION OF LITHIUM AND PILOCARPINE, THAT DISPLAYED NECROSIS WITHIN SPECIFIC STRUCTURES

Structure	Percent Damage	Structure	Percent Damage
Neocortex (all lobes)	19	Rhomboid nuc.	32
Insular cortex	29	Lateral posterior nuc. (mediorostral)	84
Piriform cortex	100	Lateral posterior nuc. (laterorostral)	55
Perirhinal cortex	23	Lateral posterior nuc.	68
Corticoamygdaloid transition	13	(mediocaudal)	
Entorhinal cortex	65	Lateral posterior nuc. (laterocaudal)	52
Dorsal endopiriform nucleus	100	Lateral dorsal nuc. (dorsomedial)	74
Ventral endopiriform nucleus	87	Lateral dorsal nuc. (ventrolateral)	83
Claustrum	35	Ventromedial nuc.	87
Caudate putamen	55	Ventrolateral nuc.	55
Globus pallidus	32	Ventral posteromedial nuc.	48
Entopeduncular nuc	25	Ventral posteriorlateral nuc.	52
Posteriormedial corticomedial nucleus	90	Gustatory nuc.	26
Posteriolateral corticomedial nucleus	84	Ventral anterior nuc.	23
Anterior cortioamygdaloid nucleus	26	Posterior thalamic nuclear group	90
Anterior amygdaloid area	03	Posterior limitans	00
Nucleus of lat. olfact. tract	00	Posterior nuc. (triangular)	06
Medial amygdaloid nucleus	35	Angular nuc.	16
Intercalated amygdaloid nucleus	03	Lateral geniculate (ventral)	00
Bed nuc. of stria terminalis	00	Lateral geniculate (dorsal)	77
Amygdaloid intramedullary gray	03	Medial geniculate (main)	25
Amygdalohippocampal area	35	Medial geniculate nuc. (dorsal)	80
Lateral amygdala nuc. (dorsolat)	51	Medial geniculate nuc. (medial)	23
Lateral amygdala nuc. (ventrolat)	64	Medial geniculate nuc. (ventral)	03
Lateral amygdala nuc. (ventromed)	71	Suprageniculate nuc.	81
Basolateral amygdaloid nuc. (anter)	19	Parafascicular nucleus	42
Basolateral amygdaloid nuc. (post)	65	Central limitans	62
Basolateral amygdaloid nuc. (vent)	48	Centromedial nuc.	55
Basomedial amygdaloid nuc.	84	Paracentral nuc.	74
Central amygdaloid *complex*	00	Substantia nigra (reticulata)	100
Thalamus		Substantia nigra (compacta)	00
Anterior dorsal thalamic	06	Hypothalamus	
Interanteromedial nuc.	13	VMH	0
Interanterodorsal nuc.	16	LH	0
Anteriomedial nuc.	74	DH	0
Anteroventral nuc.	10	Hippocampal formation	
Habenula	42	Subiculum	25
Mediodorsal nuc.	75	CA1	93
Mediodorsal nuc. (medial)	81	CA2	77
Mediodorsal nuc. (central)	83	CA3	19
Mediodorsal nuc. (lateral)	87	CA4	16
Interomedial dorsal nuc.	55	DG	32
Paraventricular nuc.	16	LS	29
Gelatinous nuc. (submedial)	42	Medial septum	00
Reuniens	97	All other telencephalon	00
Paratenial	93	All other mesencephalon	00

$n = 31$.

killed by decapitation either: 1 day ($n = 8$), 4 days ($n = 6$), 10 days ($n = 8$) or +60 days ($n = 9$) after the seizure induction. For comparison, rats that had never been disturbed (food and water ad lib, $n = 10$) or partially food deprived (to simulate the 85% body weight that is normally used in operant studies) for either 1 day ($n = 6$), 4 days (n = 5), or for 10 days ($n = 6$) were also killed by decapitation. ACTH (adrenocorticotrophic hormone) and corticosterone were assayed according to previous procedures (10).

In part C, 10 rats were tested separately. One hour after induction of the seizure each rat was shaved over the thoracic area. Two silver EEG electrodes were placed (EC2 cream) over this region and taped securely; the ground was connected to the tail. The rat was then placed within a standard restraint cage for the

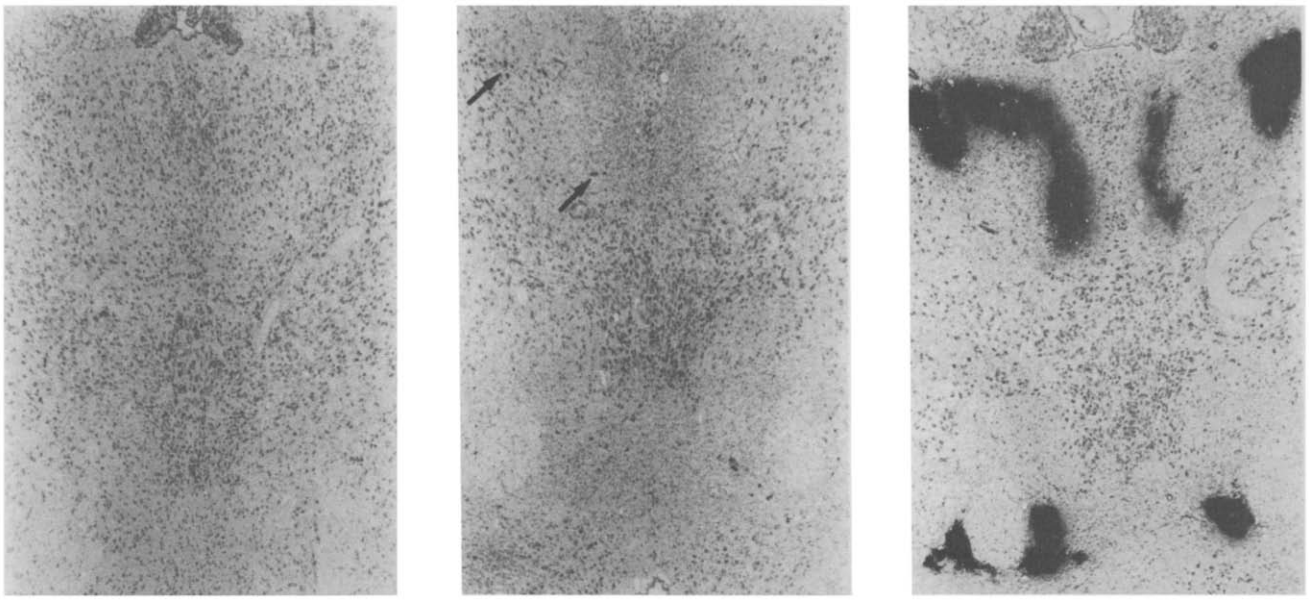

FIG. 3. Neuronal necrosis within the thalamus for rats (B,C) in which seizures had been induced about 10 days previously by a single SC injection of lithium and pilocarpine. A is a control brain (coronal sections; 28×, thionin). Note the dense Nissl stained material throughout large areas of the thalamus in C.

next 24 h. Mean heart rate for each 5-min block was determined by a small microcomputer.

In part D, a total of 20 rats in which seizures had been induced by 3 mEq/kg of lithium and 30 mg/kg of pilocarpine, using either the 4-h or 24-h interval, served as subjects. The time since the seizure induction was between 30 and 60 days. Each rat was then injected with either 2 or 3 mEq/kg of lithium and 4 h later by either 20 mg/kg or 30 mg/kg of pilocarpine. SOT was deter-

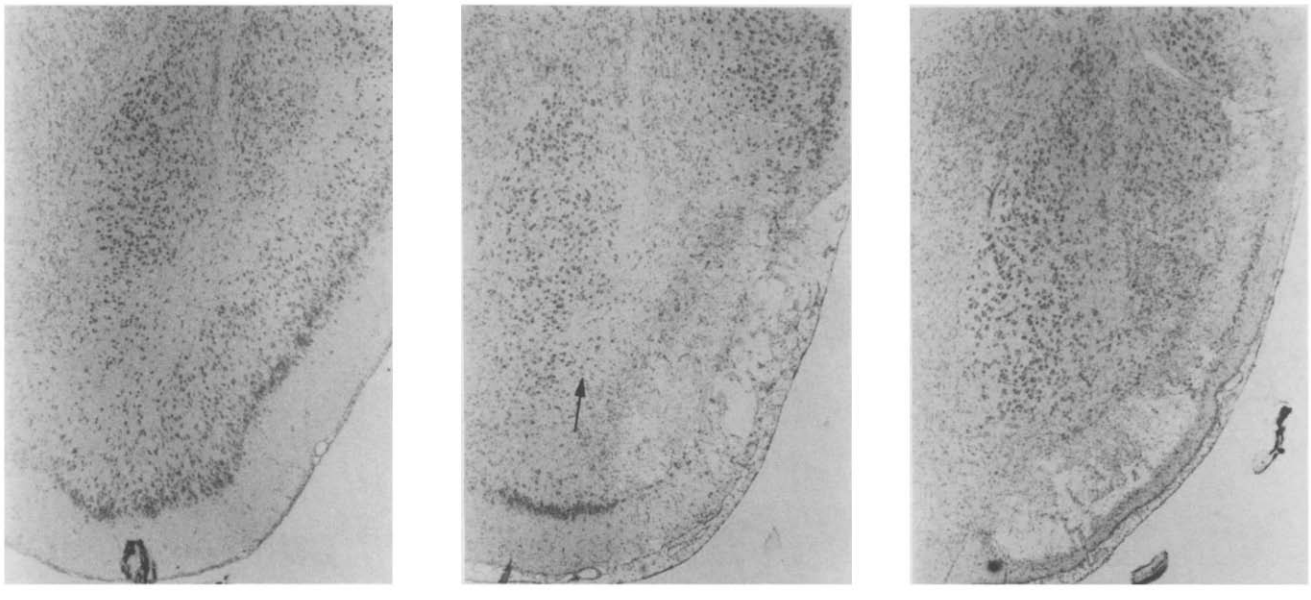

FIG. 4. Neuronal dropout within the entorhinal cortices of two rats (B,C) in which forepaw clonus had been induced by a single subcutaneous injection of lithium and pilocarpine. Note the invagination of the necrosis into the adjacent amygdaloid region in B (as indicated by arrow). A coronal section from a control rat (A) is shown as well. (29×, thionin).

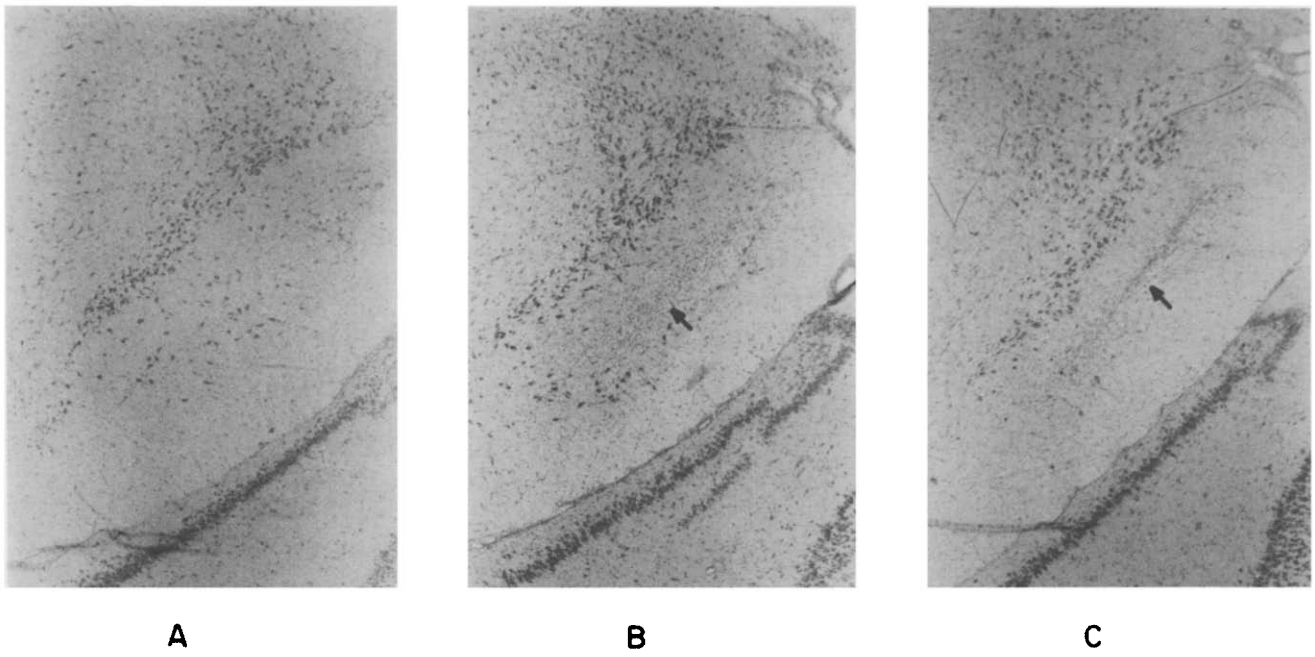

FIG. 5. The substantia nigra of a control rat (A). B and C show the selective damage (arrows) and reactive gliosis within the pars reticulata region in two rats (B,C) in which limbic seizures had been induced by lithium-pilocarpine (coronal section; 28×, thionin).

mined. In order to assess mortality, no acepromazine was injected.

In Part E, a total of 20 rats were injected with 3 mEq/kg of Li and then 4 h later with 30 mg/kg of pilocarpine; another 10 rats served as nonseizured controls. All rats were tested on a standard hot plate that was thermostat controlled at 55°C. When the rat lifted any one of its hindpaws twice and licked it, the animal was removed from the setting and the time was automatically recorded by releasing a foot-controlled timer. For 15 (10 seizure; 5 controls) of the rats, daily testing began 3 days after the seizure while for the other 15 rats testing was completed once every 3 days. The different test intervals were introduced

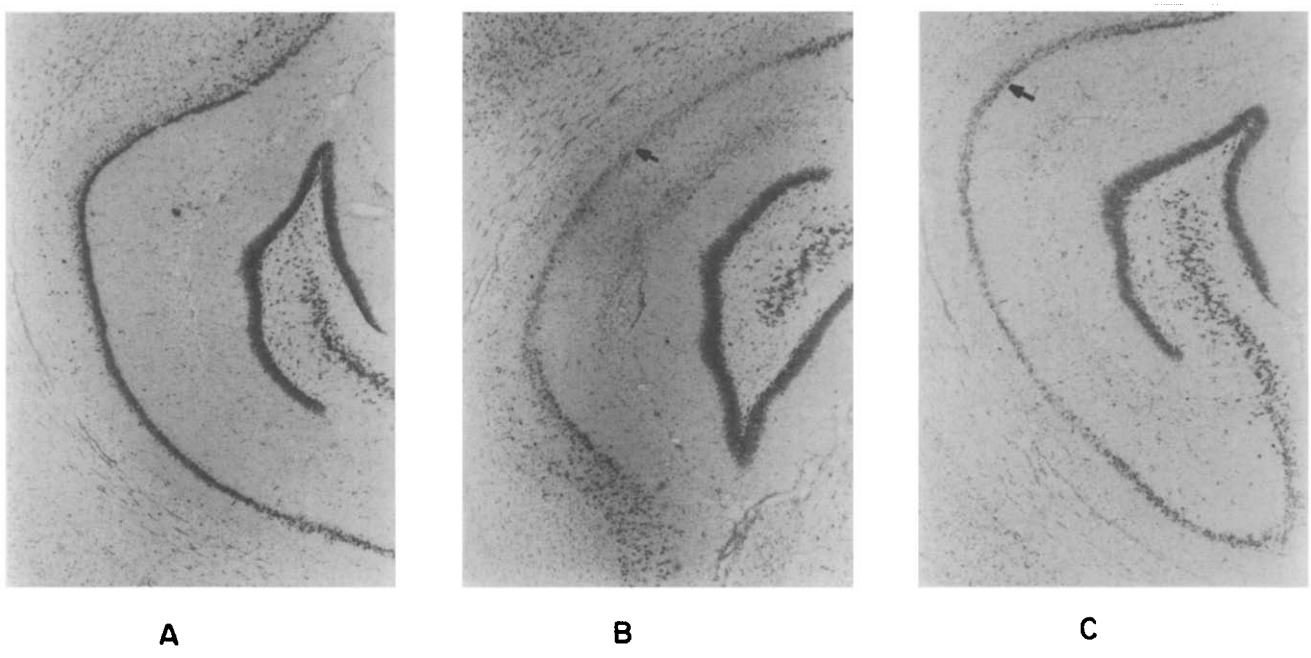

FIG. 6. Photomicrographs of hippocampal sections. (A) Normal rat. (B,C) Two rats in which seizures had been induced 30 days previously by a single SC injection of lithium and pilocarpine; note the pyramidal cell loss, especially in CA1 (arrows).

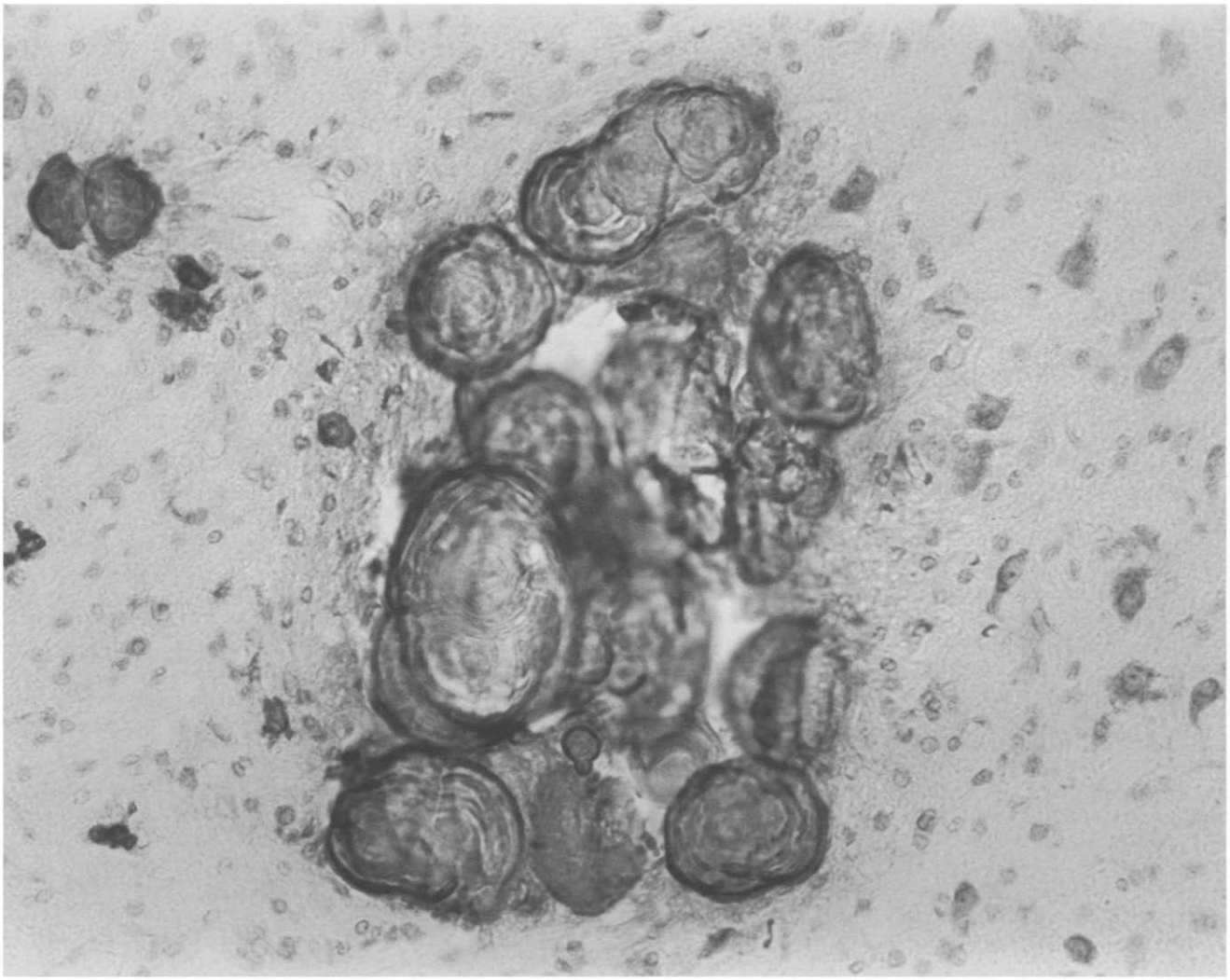

FIG. 7. Crystalline-like appearance of densely stained material within the posterior thalamic region of a rat about 70 days after seizure induction by lithium-pilocarpine. The material stains as a neutral mucopolysaccharide within which is embedded copious amounts of calcium.

in order to determine the possible contribution of (escape) learning to the response latency.

Experiment II: Aggression. In part A, 40 pairs of rats were used as subjects; seizures had been induced in all of the animals by 3 mEq/kg of lithium and 30 mg/kg of pilocarpine. The time between the two injections was 4 h for 20 of the pairs and 24 h for the other 20 pairs. There were 24 rats in part B and 21 rats in part C of this experiment. In both parts B and C each rat was housed singly. One-third of the numbers of rats served as non-seizured controls. Of the other two-thirds, one-third had received 3 mEq/kg of lithium and 30 mg/kg of pilocarpine with drug intervals of either 4 h or 24 h. Rats in parts B and C had been seizured between 14 days and 20 days earlier and had displayed the usual signs of recuperation as defined by consumption of dry chow and fluid from the water bottles.

In part A, each pair of rats was carefully examined daily for 30 consecutive days; the observations commenced 5 days after induction of the seizures. Evidence of aggression, defined as fresh or dried blood or lesions on the body (usually ears and face) was recorded for each pair. The appearance of the multiple pinpoint lesions in the tail were not considered indicators of aggression (4). If rats were reported by the animal technicians to have maintained fighting and if blood loss and scaring were considered inappropriate, the rats were housed singly and treated in compliance with the guidelines of the Canadian Council of Animal Care. The numbers of pairs of rats that displayed aggression (and that had been separated) at any time during the observation period were recorded.

In parts B and C, muricide (33) was evaluated. A single 100- to 120-day-old male Swiss albino mouse was placed in each rat's cage. The numbers of mice killed at the end of a 30-min observation period were determined. Chi-square analyses as a function of 4-h vs. 24-h seizure groups and seizure and control groups (parts B and C) were completed.

The brains of 12 of the rats (from part A) in which seizures had been induced were removed, fixed, processed, and stained within thionin or toluidine blue O (31). Six of the brains had been taken from the most aggressive rats while the remainder had been removed from the most normal (behaving) rats. The determination of damage (no, yes) within the structures that are listed in Table 1 was completed at 100 and 400 × magnification. Chi-square analyses were completed for the two groups for each

structure. Although the problem of such analyses for multiple structures (e.g., chance) was acknowledged, exploration was considered appropriate.

Experiment III: Radial maze deficits. In part A of this experiment 12 rats served as subjects. Seizures had been induced in eight of the rats 50 days before training; four of the rats had received 2 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine, while the remaining four rats had received 3 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine. The remaining four rats were controls.

In part B of this experiment, 23 rats were subjects; they had received either 3 mEq/kg of lithium ($n = 6$) followed 4 h later by 30 mg/kg of pilocarpine or 2 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine ($n = 10$); seven rats served as controls. Maze training began approximately 70 days after seizure induction. Only rats that had not displayed obvious seizures within the maze were used as subjects.

In part C of this experiment rats that had been injected with 3 mEq/kg of lithium either 4 h ($n = 7$) or 24 h ($n = 9$) before the injection of 30 mg/kg of pilocarpine began training in the RAM about 50 days after the induction of the seizures; another six rats served as controls.

In Part D of this experiment six rats that had displayed at least one limbic seizure during testing within the radial maze and four nonseizured controls served as subjects; seizure induction by typical dosages of lithium and pilocarpine had occurred about 60 days previously. Except for Part D, only rats that did not seizure within the maze were included in this experiment.

During maintained 23-h food deprivation, the rats in part A were trained over a 3–4 day period to enter each of the eight arms of the RAM (13). Each arm was baited with two 50 mg Noyes food pellets. After training had been completed, testing began. The numbers of errors, defined as reentry into an arm, and the time required to enter all eight arms was recorded. Animals were tested once per day for 8 consecutive days during the middle of the light cycle.

The animals in parts B and C of the experiment were trained with only four (alternating) arms baited within the same RAM maze; approximately half the numbers of rats were trained when arms 1, 3, 5, and 7 were baited while the other rats were trained when only the even-numbered arms were baited. Two fruit loops rather than food pellets were used as reinforcers. Each training and testing session (trial) began by releasing the rat (surrounded for about 10-s by a plastic cylinder) from the center of the maze.

Testing began once the rat had been shaped to proceed to the end of the arms and obtain the rewards. The numbers of entries into an arm that was never baited were defined as reference (long-term) memory errors, while the numbers of reentries into an arm that had been baited that session were called working (short-term) memory errors. Each animal was tested until all baited arms had been successfully entered. There were eight sessions (trials); the intertrial-interval was 2 days.

In part D of this experiment, rats that been given 8 to 10 days of experience in the baited/nonbaited paradigm were tested daily for 8 days. Every second day, 1.5 h before the daily session, all rats received 10 mg/kg of carbamazepine (Tegretol) intraperitoneally; on alternative days they were injected with saline. For each rat, the numbers of working and reference errors that were displayed during days in which the Tegretol had been injected were subtracted from the working or reference errors displayed on days when the placebo had been received.

Experiment IV: Conditioned taste aversion. In part A, a total of 27 rats were selected as subjects; seizures had been induced 20 days previously. Eleven of the rats had received 2 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine and seven of the rats had received 3 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine; for the remaining nine rats, five had received 3 mEq/kg of lithium only while four had received 30 mg/kg of pilocarpine only (because there were no statistically significant differences between these groups they were combined and served as drug controls for subsequent analyses). In part B, 18 rats were seizured by 3 mEq/kg of lithium and 30 mg/kg of pilocarpine; the time between the injection of these substances was 4 h for nine animals and 24 h for the others. Ten animals served as normal controls. Rats were housed singly and administered the recovery procedure; all rats returned to dry food and normal fluid access (bottles) within 15 days. Fifteen days later (when all animals had returned to dry food and water bottle intake) rats began a restricted fluid intake schedule.

Each rat was allowed access to fluid for only 20 min per day (32). The volume (ml) of water consumed during 5 successive days was recorded. On day 6, the rats were given a 10% solution of condensed milk. After 3 more days of water during the fluid sessions, a 10% solution of sucrose was made available. Within 30 min each rat was injected IP with 10 ml/mg of 0.15 *M* lithium chloride (pairing). Following 2 more days of water, sucrose was presented again (test). Three measures of consumption were calculated for these experiments:

1. the ratio of milk consumed relative to the mean of the water intake of the previous 3 days,
2. the ratio of sucrose consumption on the pairing day relative to the mean water consumption of the previous 2 days, and
3. the ratio of consumed sucrose during the test day compared to the consumption on the pairing day (CTA score).

These measures were correlated with SOT and the percentage of body weight 5 days after the seizure compared to the baseline. SOT and percent body weight were considered potential indices of the severity of the seizure-induced brain damage.

Experiment V: Possible conditioned postinduction seizure displays. All rats had received 3 mEq/kg of lithium chloride followed 24 h later by 30 mg/kg of pilocarpine. In part A, a total of 40 rats had been injected 3 mEq/kg of lithium and 30 mg/kg of pilocarpine 4 h later. They were restrained (36,39) for 4 to 6 min (for blood collection via the tail vein) just before the pilocarpine injection and again on the fifth and tenth day after the seizure induction. The numbers of rats that displayed spontaneous classic seizures (forelimb clonus and falling) during the transport from the home cage to the restraint room were recorded.

In Part B, six to eight pieces of rat chow were placed on the bottom of the wire cages of 12 cages (18 males, 18 females) of rats (three/cage) at precisely 1600 h for 25 consecutive days (postseizure days 25 to 50). The occurrence of spontaneous myoclonic seizures and rearing were recorded per group (because there were no rate of seizure differences between sexes, the results were combined). In part C, the records of 20 rats (not included in Experiment III) who were tested daily in the RAM and who had displayed the limbic seizure pattern were examined. The incidence of seizures per day was recorded. In Part D, 10 rats that had been seizured 10 days previously were placed in an open 37 cm by 30 cm by 18 cm (deep) plastic container every second day for 23 days. The occurrence of a limbic seizure within 3 min was recorded for each rat.

RESULTS

Experiment I

The percentage of rats per group that were alive after 3 days were: 10 mg/kg acepromazine: 50%, 25 mg/kg of acepromazine:

80%, 10 mg/kg chlorpromazine: 0% and 25 mg/kg of chlorpromazine: 20%; all of the control rats died. Chi-squared analysis, with the two dosages of each drug combined, indicated a statistically significant (chi-square = 12.90, $p < 0.001$) prophylactic effect by the acepromazine.

There were no statistically significant differences, $F(7, 50) = 0.82$, $p > 0.05$; chi-squared from Kruskal–Wallis also $p > 0.05$, in ACTH levels (trunk blood) between control rats and rats that had been either food deprived (FD) for either 1 day, 4 days, or 10 days or rats in which seizures (S) had been induced either 1 day, 4 days, 10 days, or more than 50 days previously (grand mean = 291 pg/ml, SD = 329 pg/ml). However, a one-way analysis of variance demonstrated a significant, $F(7, 50) = 15.73$, $p < 0.001$, corticosterone difference. The groups, means (ng/ml) and standard deviations (in parentheses) were: control: 149 (129), FD + 1 day: 133 (90), FD + 4 days: 436 (250), FD + 10 days: 456 (230), S + 1 day: 745 (255), S + 4 days: 111 (92), S + 10 days: 86 (77), and S + 50 days: 133 (128).

Post hoc analyses indicated that the major source of the group differences occurred between rats that had been food deprived for 4 or 10 days or that had been seizured the day before relative to all other groups. Two-way analyses of variance as a function of time (+1, +4, +10 days) and group (food deprived vs. seizure) revealed statistically significant interactions for the relative weight loss, $F(2, 34) = 3.85$, $p < 0.05$, and corticosterone levels, $F(2, 34) = 26.92$, $p < 0.001$, but not the ACTH levels, $F < 1$. The interactions were due to the greater weight loss (85% baseline) and elevated corticosterone levels in the food-deprived rats compared to the seizured rats (who had returned to baseline weights) at day 10.

During the 24-h period of constant cardiac monitoring, six of the rats died. In all cases the EKGs were characterized by progressive periods of brief bradycardia and arrythmia. The most severe incident was associated with the rat's death. Figure 8 shows an example of this profile. Seizure onset time (SOT) of rats in which lithium-pilocarpine had been injected a second time, after recuperation from the first episode, exhibited a conspicuous biomodal distribution. Either the rats (45%) displayed overt seizures within 10 min of the pilocarpine injection or did not seizure during the 70-min observation period (and showed no evidence of seizuring, e.g., weight loss during subsequent days). There was a 95% survival rate for the rats who received the second lithium-pilocarpine injection (including those that displayed overt forelimb clonus) even though they had not received acepromazine.

The foot-lick latency curve for the hot plate experiment is shown in Fig. 9. Each point is composed of the mean of all of the rats that had received the lithium-pilocarpine treatment. The mean and SD for the control rats that were tested daily over a comparable period was 20 and 5 s, respectively. Rats in which seizures had been induced approached the values of controls within 20 days.

Experiment II: Aggression

During the 30-day observation period, 80% of the pairs of rats that had received 4-h interval treatment and 15% of the pairs that had received the 24-h interval lithium-pilocarpine treatment displayed indications of aggression (chi-squared = 10.89, $p < 0.001$). Body lesions first began about 10 days after the induction of the seizure and were still evident at the end of the observation period. The numbers of indicators (70% of the 4-h animals) peaked and approached an asymptote between postseizure days 15 and 20. Whereas five of the 4-h pairs were separated for humane reasons, none of the 24-h interval pairs required separation.

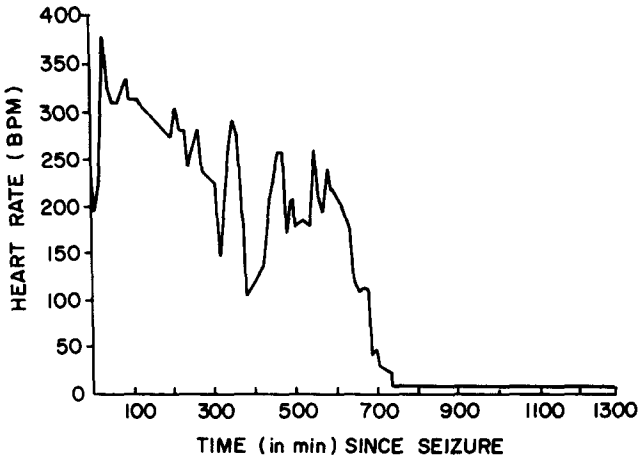

FIG. 8. Heart rate (beats per min) for a rat (in mild restraint) during the subsequent minutes that followed the induction of the limbic seizures by lithium-pilocarpine. The rat had not received acepromazine; death occurred about 10 h after the seizure onset.

The percent of rats that killed mice in part B for the 4-h, 24-h, and control treatments were 38%, 25%, and 38%, respectively (chi-square = 0.37, $p > 0.05$); for part C, the muricide incidence for these groups was 57%, 43%, and 43%, respectively (chi-square = 0.38, $p > 0.05$). There was also no statistically significant difference between muricide incidence between the two experiments (chi-square = 0.96, $p > 0.05$).

Chi-squared analyses of the qualitative histological examination of the brains from the six most aggressive and the six least aggressive rats demonstrated statistically significant distributions ($p < 0.05$) for only four structures. The aggressive rats displayed 100% damage within the claustrum and cingulate gyrus relative to the least aggressive rats (no damage). Relative to the nonaggressive rats who showed no damage within the triangularis of the posterior nucleus and the centromedial nucleus of the thalamus, 66% of the aggressive rats displayed damage in these two structures. All other areas (for both groups of rats) exhibited incidence of necrosis that were comparable to reference values (Table 1).

Experiment III: RAM

Figure 10 shows the total numbers of errors (reentry into arms) and the time required to reach criteria (entry to all eight arms) for the three groups in part A of this experiment. Two-way analyses of variance, with one level repeated (eight sessions), for the three treatments (controls, 2 mEq/kg-Li 30 mg/kg pilocarpine and 3 mEq/kg Li-30 mg/kg pilocarpine) indicated a significant treatment, $F(2, 9) = 6.19$, $p < 0.02$, difference for errors; neither the day effect nor the day by treatment interaction was significant. Post hoc analysis indicated that the 3 mEq/kg-Li 30 mg/kg pilocarpine group displayed more errors than the other two groups. One-way analyses of variance indicated that the significant differences between the control and treatment groups (for both errors and time) did not appear until the sixth session.

The mean numbers of working and reference errors per session for another group of the treated and control rats (part B) are shown in Fig. 11. A three-way analysis of variance with two levels repeated (working vs. reference memory; eight sessions) and treatment revealed a significant, $F(2, 19) = 18.69$, $p < 0.001$, group difference. Post hoc analyses indicated that this was due

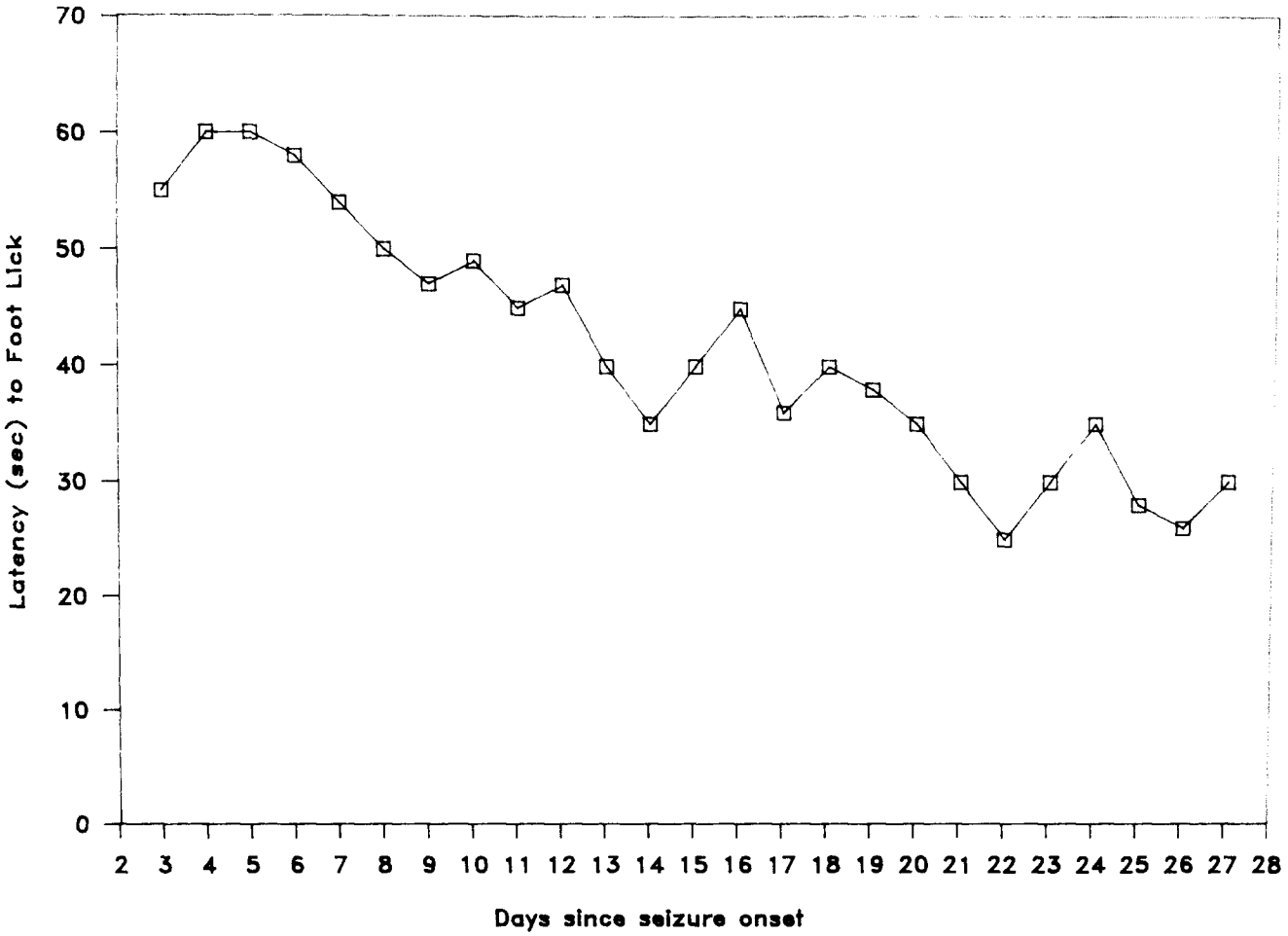

FIG. 9. Inference of recovery from the seizure by the latency (maximum 60 s) for the foot lick response in a hot plate (55°C). Latency over time is indicated and asymptotes at about 20 days postseizure.

primarily to the poor performance by the two seizured groups (2 mEq/kg Li and 3 mEq/kg Li) relative to controls. There were no statistically significant contributions from memory type, session, or the interaction of the two.

To verify that the controls were responding appropriately, repeated measure analyses were completed for these rats for the working, $F(7, 35) = 3.61$, $p = 0.005$, and reference, $F(7, 35) = 8.98$, $p < 0.001$, memory errors over time; both domains displayed the learning effect. However, there was no significant change over the eight sessions for the seizured rats (groups combined) for either working, $F(7, 105) = 0.56$, $p > 0.05$, or reference memory, $F(7, 105) = 0.47$, $p > 0.05$, errors.

The mean numbers of working and reference memory errors for nontreated rats and for rats that had received the 3 mEq/kg Li and then 30 mg/kg of pilocarpine either 4 h or 24 h later (Part C), are shown in Fig. 12. Three-way analysis of variance with two levels repeated demonstrated statistically significant group differences, $F(2, 19) = 54.04$, $p < 0.001$]. Post hoc tests indicated that the 24 h interval rats displayed more overall errors (working and reference combined) than the 4-h group; both of these groups displayed more errors than did the controls.

There were no statistically significant differences between the numbers of working or reference memory errors displayed by the groups, $F(1, 19) = 2.18$, $p > 0.05$, and no significant treatment by type of memory interaction, $F(2, 19) = 1.57$, $p > 0.05$. None of the other repeated measures or interactions were statistically significant. Whereas the controls displayed the expected learning curve, as defined by less errors over time, the two groups of treated rats did not display any evidence of learning, $F(7, 133) = 0.95$, $p > 0.05$.

The means and standard deviations (in parentheses) for the differences between the numbers of short-term (working) and long-term (reference) errors on days in which the placebo (Experiment III, part D) had been given compared to days in which the anticonvulsant Tegretol had been given were: 0.2 (0.6), 0.4 (0.4) for the nonseizured rats and 0.9 (2.5) and 2.4 (1.9) for the seizured rats, respectively. Two-way analyses of variance with one level repeated (difference scores for drug and nondrug days for short-term and long-term memory) and treatment (nonseizure, seizure) demonstrated that more long term memory errors were displayed, $F(1, 8) = 15.50$, $p < 0.01$, than short-term memory errors. The significant, $F(1, 8) = 6.33$, $p < 0.05$, treatment by memory-type interaction was due to the relatively greater numbers of long-term memory errors (i.e., on Tegretol days) displayed by the seizured rats compared to the nonseizured rats. During the 8 days of testing none of the six rats in which seizures had been induced 60 days previously by the lithium/pilocarpine treatment displayed any discernable myoclonic seizures within the maze.

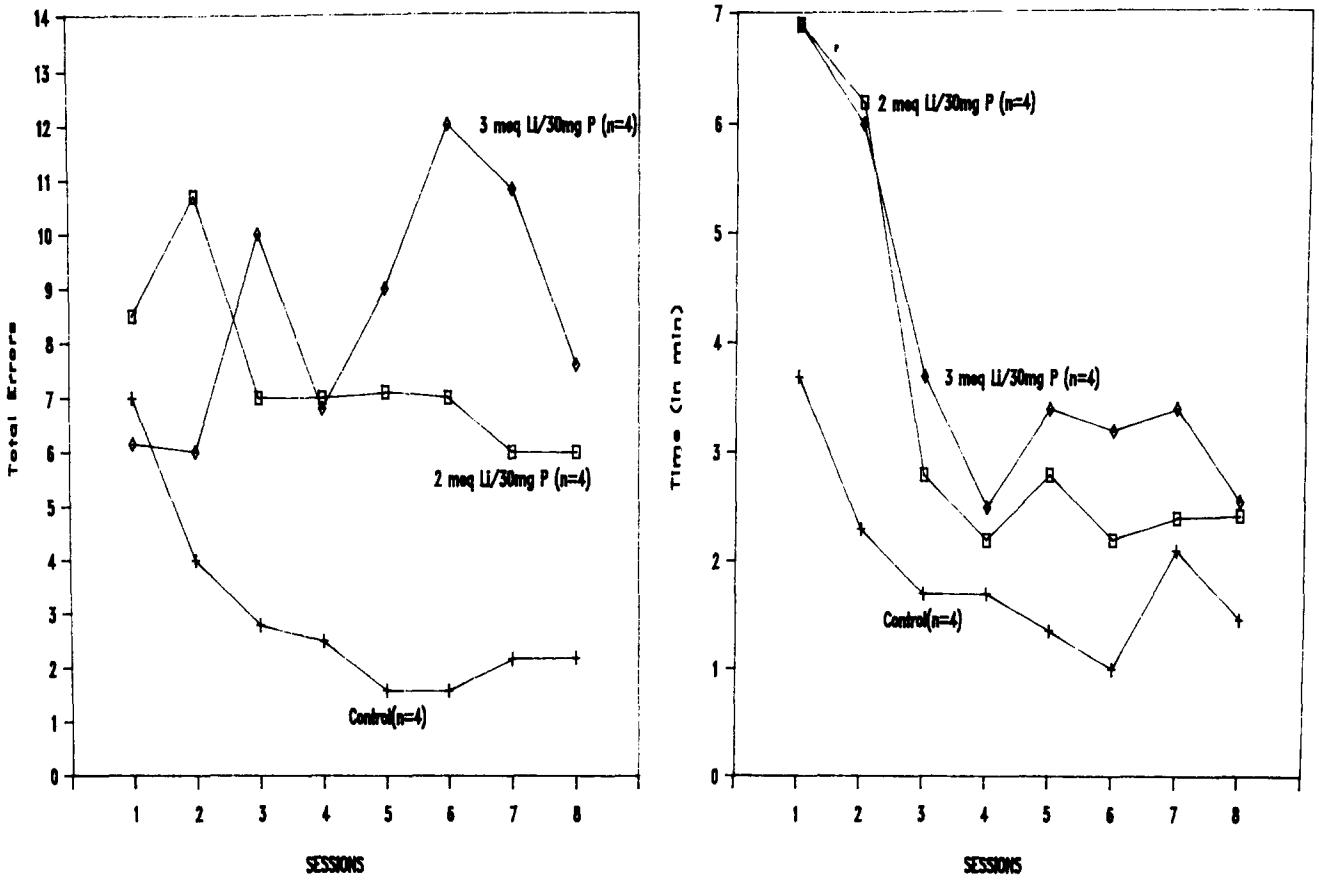

FIG. 10. The left panel shows the mean numbers of total errors per session in a radial arm maze (RAM) for control rats or rats that had received either 2 mEq/kg or 3 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine about 50 days previously. The right panel indicates the time (in minutes) required to achieve criterion (entry into all eight arms) per session for each group.

Experiment IV: CTA

The milk/water ratio, sucrose consumption on pairing day/water ratio, and CTA scores for the groups that received 3 mEq/kg of lithium-30 mg/kg of pilocarpine, 2 mEq/kg of lithium-30 mg/kg of pilocarpine (both 4-h intervals), and the control treatments are shown in Fig. 13A. One-way analyses of variance, all $F(2, 24)$ with Tukey's post hoc tests set at $p < 0.05$ indicated that both seizured groups displayed significantly more milk consumption than controls, $F = 8.89$, $p < 0.001$. The significant difference in CTA scores, $F = 9.54$, $p < 0.001$, was due primarily to the absence of CTA for the 3 mEq/kg lithium group compared to the controls. There were no significant group differences for the relative amount of sucrose consumed during the paring day.

Two-way analyses of variance with one level repeated (milk/water ratio, pairing sucrose/water ratio and CTA ratio) and the three treatments demonstrated the main effect, $F(2, 24) = 12.02$, $p < 0.001$; there was a statistically significant difference between the ratios, $F(1, 24) = 20.90$, $p < 0.001$, and a statistically significant ratio by treatment interaction, $F(2, 24) = 4.16$, $p < 0.01$. It was due primarily to the presence of CTA in the group that had been seizured by 2 mEq/kg of lithium even though their relative milk consumption did not differ from the 3 mEq/kg lithium group. Covariance for final (CTA) body weight, $F(1, 23) = 1.79$, $p < 0.05$, was not significant statistically and did not change the strength of the treatment effects.

SOT was significantly ($p < 0.05$) correlated ($r = -0.49$) with the milk consumption ratios but not with either volume of sucrose consumed during pairing ($r = -0.21$) or CTA ($r = -0.06$). The percent body weights 5 days after seizure induction was 90% for the 2 mEq/kg, 83% for 3 mEq/kg rats, and 102% for the controls. For the seizured groups (combined) only the percent body weight was correlated -0.68 ($p < 0.01$) significantly with the milk/water ratios but not with the pairing sucrose/water ratio (-0.10) or CTA scores (-0.29).

The milk/water ratios, sucrose (pairing)/water ratios, and CTA scores for the rats (part B) that been seizured with either the 4-h or 24-h interval and their controls are shown in Fig. 13B. One-way analyses of variance (all $df = 2, 25$) and Tukey's post hoc analyses showed that the group that had been seizured with the 4-h procedure drank significantly, $F = 16.15$, $p < 0.001$, more milk (×2 water consumption of previous days) than rats in which seizures had been induced by the 24-h procedure; they, in turn, drank relatively more milk than controls. Whereas there were no group differences, $F = 3.50$, $p > 0.05$, with respect to sucrose consumption on the pairing day, both groups of seizured rats displayed, $F = 5.84$, $p < 0.01$, less CTA than the controls; there was no difference in CTA between the two seizured groups.

A two-way analyses of variance with one level repeated (milk/water ratio, pairing sucrose/water ratio and CTA ratio) and one level not repeated (treatment) revealed significant treatment differences, $F(2, 25) = 18.01$, $p < 0.001$, ratio differences, $F(2, 51)$

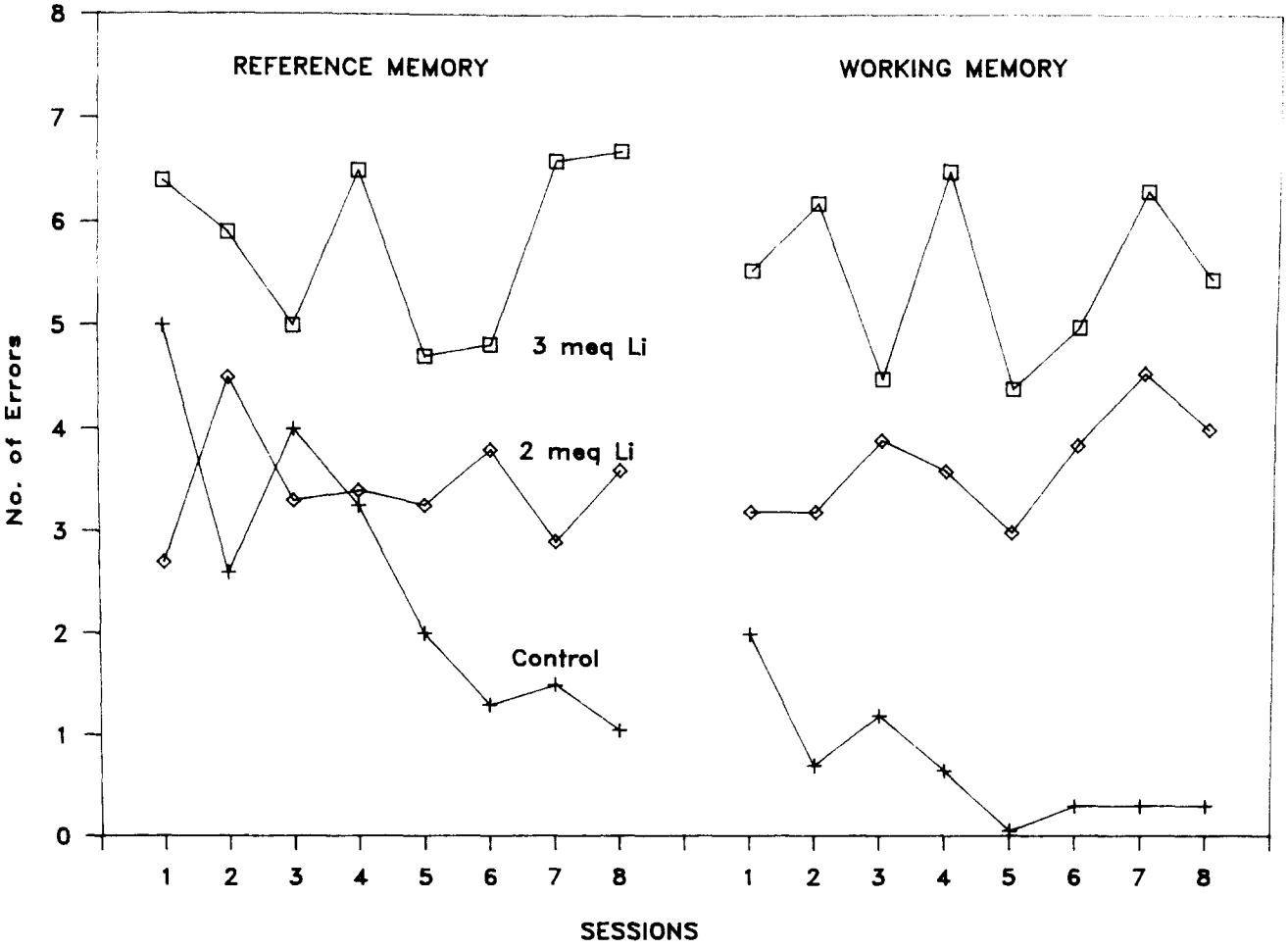

FIG. 11. Mean numbers of reference memory and working memory errors within the RAM for control rats and for rats that had received either 2 mEq/kg or 3 mEq/kg of lithium followed 4 h later by 30 mg/kg of pilocarpine about 50 days before maze testing.

= 26.45, $p < 0.001$, and a treatment by ratio interaction, $F(2, 51) = 6.01$, $p = 0.01$. The latter was due to the greater relative milk consumption for the 4-h interval seizured group relative to the 24 h seizured group even though they displayed equivalent CTA. Covariance for the final body weight at the end of the experiment, $F(1, 24) = 0.07$, $p > 0.05$, did not alter the significance.

One-way ANOVAs with Tukey's post hoc ($p < 0.05$) indicated that the 24-h group weighed [430 (50) g] less than the 4-h injection interval [452 (32)] and control [481 (29)] groups, $F(2, 25) = 5.64$, $p < 0.01$, who did not differ significantly from each other; however the percent body weight 5 days after the seizures had been induced were comparable for the two seizure groups [85(7)%; 85 (12)%, respectively] and significantly different, $F(2, 25) = 3.29$, $p < 0.05$, from the controls [100% (2)].

The percentage of body weight 5 days after the seizure (about 20 days before testing) was significantly correlated with the amount of milk consumed ($r = -0.50$) and the milk/water ratio (−0.66) but not with either sucrose consumption (−0.07) on the test day or the CTA ratio (−0.33). However, SOTs were significantly correlated with the percentage of body weight 5 days later (0.46), milk consumption (−0.49), and the milk/water ratio (−0.51) but not with either sucrose consumption on the pairing day (−0.21) or the CTA score (−0.07).

Experiment V: Possible Learned Seizure Display

In part A, 28% of the rats spontaneously seizured between the home cage and test room on day 5, while 35% displayed spontaneous forelimb clonus on day 10; 71% of the rats that displayed these seizures exhibited them on both days. In order to facilitate comparison (part B) for rats that displayed seizures following introduction of food to the cage at the same time each day, the percentage of the population that displayed an overt seizure per session was calculated. The cumulative percentage of seizures for the three experiments are shown in Fig. 14. Record A represents the cumulative seizure incidence for rats whose floor feeding schedule (despite ad lib food in the hoppers) was synchronized to a precise daily time.

An inflection in the rate of seizure incidence is clearly evident within 5 days of the synchronized feeding; seizure incidence during the first 5 days was not different from the 10 days of random baseline observation. The rate of seizure episodes within the group was relatively consistent for the observation period.

The cumulative record for seizured rats that were maintained on 23-h food deprivation and tested daily in the radial maze is shown in record B. All of these rats (not used in Experiment III) seizured at least once; 45% of them seizured either within the cage during transport to the maze room or within 1 min of

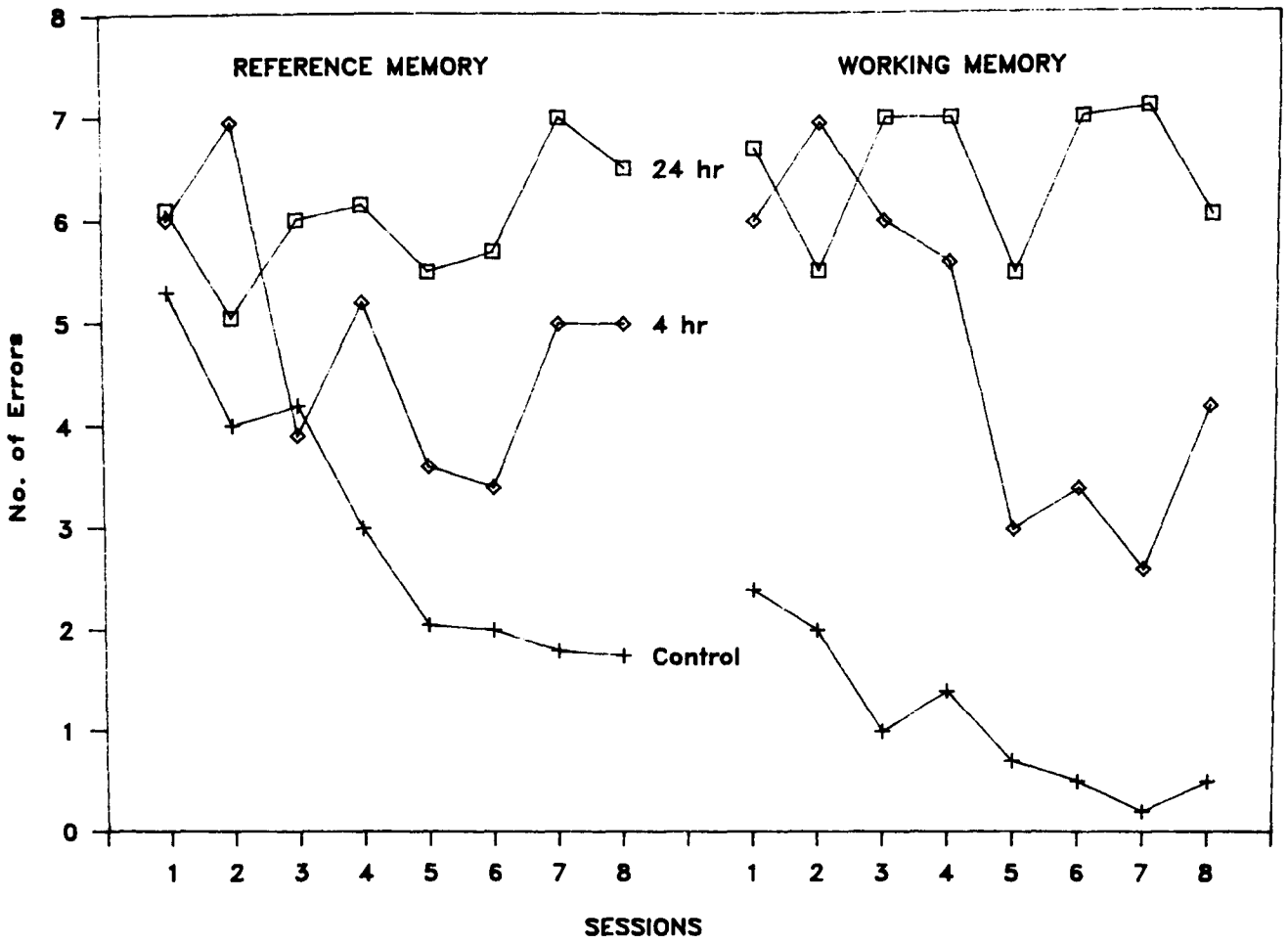

FIG. 12. Mean numbers of reference memory and working memory errors within the RAM for control rats and for rats that had received 3 mEq/kg of lithium and 30 mg/kg of pilocarpine either 4 h or 24 h later; the rats were tested about 50 days after this treatment and subsequent seizure induction.

placement within the RAM. The cumulative record indicates that the rate of seizuring for the group was constant during the 10 days of maze testing.

The cumulative record for individual, satiated (food and water ad lib) rats that had been placed in the open field box once every 2 days for 10 sessions is shown in record C. The habituation profile, unlike the displays noted in parts A and B, was conspicuous.

DISCUSSION

The change in a particular response following a singular, strategic lesion has been the traditional method by which neurobehavioral correlates have been investigated. However, clinical populations often display multifocal lesions that exhibit insidious properties and affect several classes of behavior. We have been interested in the effects of such multifocal, temporally increasing brain damage upon the organism as a whole behavioral system. By studying the potential interactions between compromised brain regions upon a variety of tasks that infer general processes, such as motivation, learning, and attention, a clearer and more clinically applicable understanding of brain function might emerge.

Such an approach should compliment and should extend the general knowledge of brain and behavioral correlates that have been determined by surgical manipulations. For example, memory deficits within the radial arm maze have been reported in rats following selective damage of either the dorsomedial thalamus (41), caudate (28), frontal cortices, amygdala, or hippocampus (19); from this perspective, the relative importance of these structures for this task can be difficult to evaluate. On the other hand, Harrigan, Peredery, and Persinger (14) demonstrated that the extensiveness of the damage only within the mediodorsal thalamus was strongly (rho = 0.80) correlated with the numbers of task errors, despite quantitatively similar and concomitant damage within the amygdala, basal ganglia, insular cortices, and other thalamic nuclei.

Although the biochemical and neuroelectrical sequelae to single, subcutaneous injection of lithium chloride followed 4 h to 24 h later by pilocarpine have contributed to the understanding of limbic seizures (15), the subsequent behavioral changes have not been pursued. This has been due, in large part, to the significant mortality (46) that normally occurs within 24 h to 72 h after the induction of the seizures. The results of our experiments indicate that mortality is reduced when the neuroleptic acepromazine is injected soon after the onset of forelimb clonus

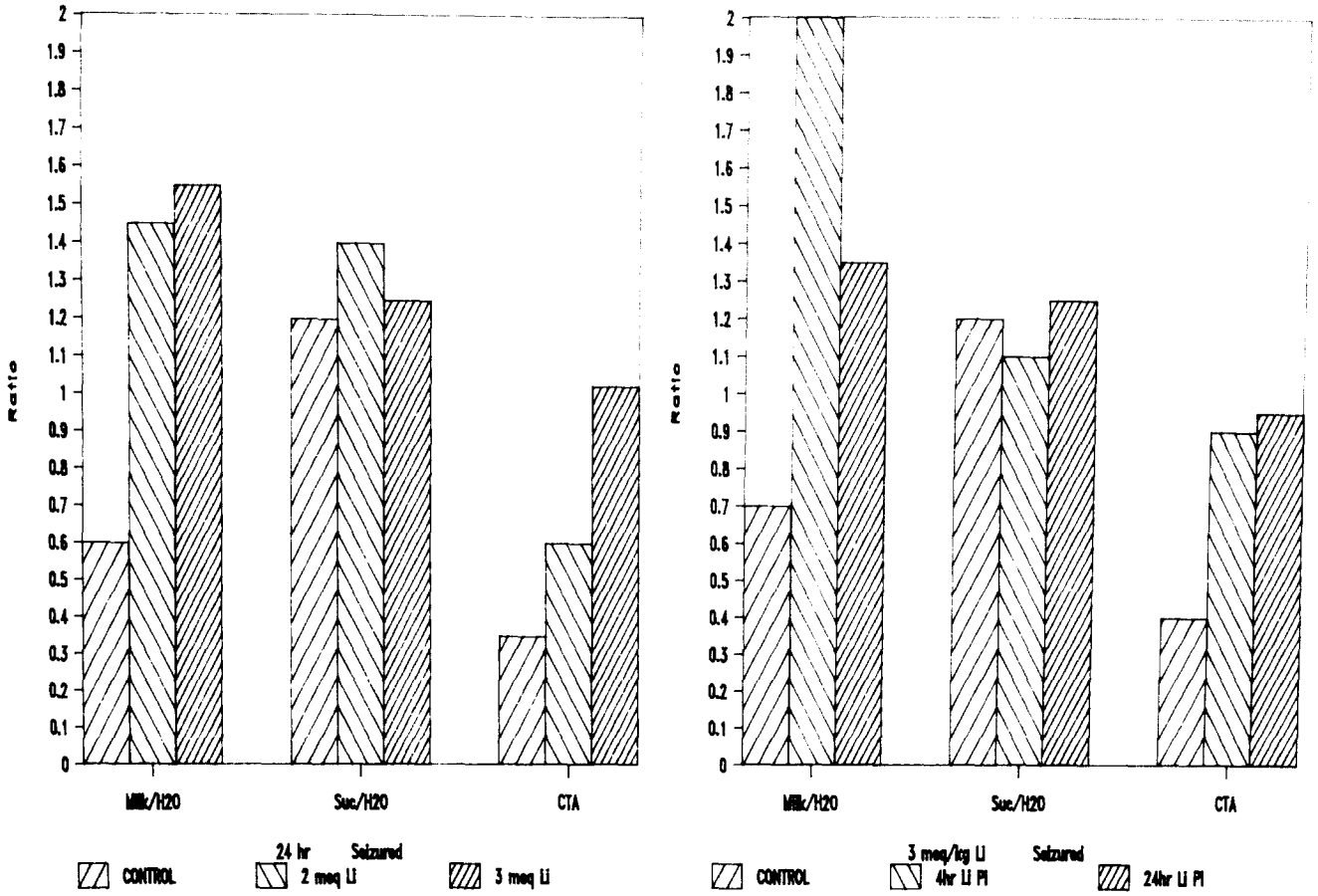

FIG. 13. Left panel: ratio of consumption (ml) of milk/water, 10% sucrose/water and test/pairing sucrose consumption (conditioned taste aversion) for control rats or rats that had received either 2 mEq/kg or 3 mEq/kg of lithium 24 h before 30 mg/kg of pilocarpine about 30 days before training. Right panel: similar ratio measures for another group of animals that were trained and tested about 30 days after receiving 3 mEq/kg of lithium followed either 4 or 24 h by 30 mg/kg of pilocarpine.

and if the appropriate postseizure appetitive procedures are instituted.

Classical biochemical indices of distress, such as blood ACTH and corticosterone, indicated that the rats in which the limbic seizures had been induced were not exhibiting chronic endocrine disruption. The levels of ACTH in rats that were killed one or more days after the seizure induction, and acepromazine treatment did not differ from that of controls or food-deprived rats. The magnitude of the transient elevation of corticosterone that was measured during the first day after the seizure induction (but not subsequently) was comparable to that evoked by mild to moderate food deprivation (85% of free-feed body weight). Within 10 days after the seizures had been induced the rats had regained their baseline body weights.

Careful examination of the pattern of behaviors that accompany and follow the forelimb clonus can be used to infer alterations in activity within multiple brain sites. The hypothermia, aphagia, adipsia, and anomalous oral movements (mouthing rather than licking the mush bowl) that occur during the first few days after the seizure was induced (even though overt displays may not be obvious) would suggest disruption of hypothalamic input from the amygdala or ventral striatum rather than damage within the hypothalamus itself. The incessant gnawing behavior (sufficient to produce severe lesions in the tails of cage mates) could be considered a form of orofacial dyskinesia; it indicates the lack of normal inhibition of the substantia nigra reticulata upon the adjacent, dopamine-containing compacta region (5).

Neurohistological assessment of the brains of these rats is compatible with these observations and inferences. The first major structures to be decimated following the seizure induction include the corticomedial amygdala, the supragenicular nucleus, and mediodorsal nucleus of the thalamus, the substantia nigra reticulata, the caudate (face region), and the piriform-entorhinal cortices. Although traditional neuroclinical interpretations would support the association between these structures and behaviors, other regions within the diencephalon and subcortical telencephalon are affected as well. Within the context of this approach, definite conclusions must await the results of canonical correlations between quantitative measures of the different components of these behaviors and the quantitative indices of the multifocal brain damage.

The temporal dimension of this model of brain damage was clearly evident from both neurohistological and behavioral observations. The positive correlation between the relative size of the lateral ventricles and the time since seizure induction is strong support for a progressive, degenerative process (37). That structure-specific damage progresses at variable rates was shown, for example, by the gradual accumulation of dense, cell-like debris within the dorsomedial, posterior, and midline nuclei of the thalamus, while other nuclei were spared.

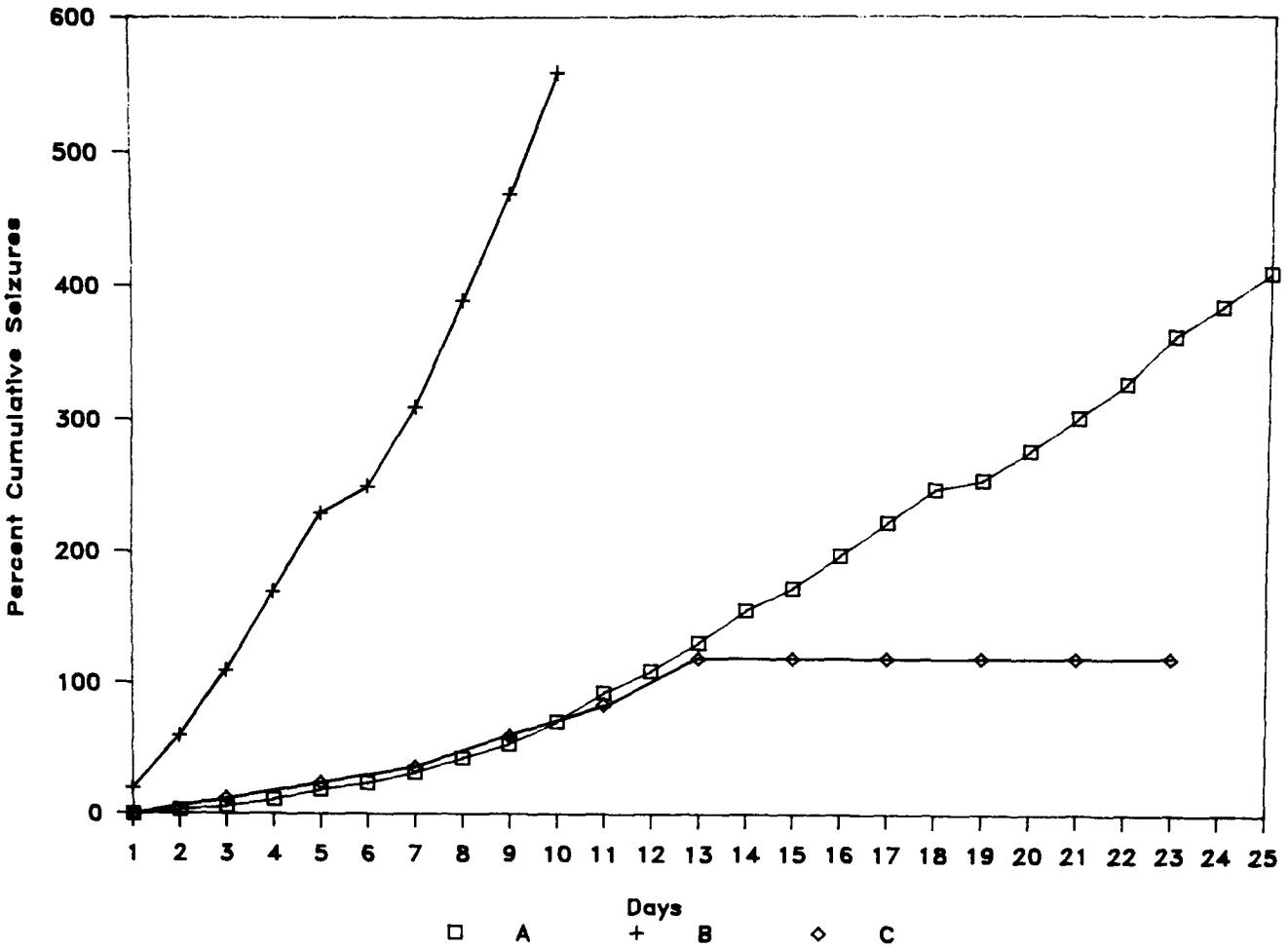

FIG. 14. Percent cumulative spontaneous seizures (primarily standing, forepaw clonus, falling and fear reactions) over days in different contexts for different groups of rats in which the single injection of lithium-pilocarpine had been given. A (squares) indicates the record for rats during daily synchronized feeding; B (pluses) indicates spontaneous seizures while being tested within a radial maze, and C (diamonds) shows repeated presentation of (food) satiated subjects to an open field setting.

Postseizure time (and presumably brain-damage)-dependent emergence of behaviors was also observed. Whereas qualitatively the amount of gnawing corresponded to the increased gliosis within the substantia nigra reticulata, the emergence of intense conspecific aggression between male rats (48) appeared about 2 weeks after the seizure induction by the lithium and pilocarpine injection. The delayed emergence of intramale aggression, without concomitant increase in muricide, is compatible with a progressive spread of damage within the amygdala. One could argue that as inhibitory nuclei within the amygdala were decimated by excitation-induced cell death, adjacent nuclei were disinhibited, and intractable but specific aggressive patterns were displayed.

Although multifocal and extensive, the distribution of the necrosis within the amygdala and the type of aggressive behavior that was (or was not) displayed by these rats are compatible with other studies. Most researchers (2,16) have found that damage to the central amygdala complex was essential for the suppression of muricide in rats. This structure was never damaged in rats in which seizures had been induced by lithium-pilocarpine, and the frequency of muricide for these rats did not differ from controls in two separate studies.

If we assume that the extent of damage is correlated with an earlier onset of protracted neuronal excitation, then the behaviors that are associated with stimulation of the corticomedial amygdaloid region would have initially dominated and (as insidious necrosis evolved) be replaced by behaviors associated with the traditionally antagonistic basolateral group (11). This spread of damage and concomitant excitation could explain the initial depression; adipsia, and aphagia that was followed within 2 weeks by defensive (territorial) aggression, excitement, and disinhibited performance. Considering the significant associations between these amygdaloid nuclei and the ventromedial and lateral hypothalamus, such hypothalamic-like behaviors are predictable.

There were also phasic behaviors that could reflect the principles (9) of focal metabolism within the brains of people who display complex partial epilepsy. Positron emission tomography has shown that epileptic foci are usually hypermetabolic during ictal intervals but hypometabolic during interictal periods. The rats in this study displayed a similar pattern for weeks to months after they had recuperated physiologically from the initial seizure induction. Within 30 s of the onset of spontaneous forepaw clonus, the rats became extremely fearful; escape behaviors, often

culminating with collision into the walls of the home cage, occurred.

However most of the time, during interictal periods, the same animals demonstrated a marked reduction in fearfulness and reduced neophobia; their enhanced exploratory behavior in novel situations was qualitatively conspicuous. The repeated, spontaneous occurrences of these kindling-like seizures indicate that a critical number of cells and their epileptogenic processes were still present (6), despite the significant brain damage within the limbic subcortex and cortex. In light of Gale's concept (12) that the substantia nigra reticulata is a final gateway that prevents the generalization (and, hence, overt symptoms) of afterdischarges, the actual size of the unstable electrical focus within the amygdala-piriform system could be smaller than normal.

The effects of the seizure-induced brain damage upon two traditional models of learning: the radial arm maze (RAM) and conditioned taste aversion (CTA), both replicated previous results and complimented current views concerning neurostructural pathways. Significant deficits were observed in both classes of behaviors, even though the rats appeared to have recuperated from the adverse correlates of the initial seizure. Qualitative observations of food and water consumption and objective measures of nociceptive thresholds (hot plate foot lick latency) suggested that the rats had returned to control levels.

However, rats in which the seizures had been induced did not display reductions in RAM errors despite a gradual reduction in the time required to achieve criterion. Differential assessment of reference memory and working memory indicated that both were adversely affected. Unlike normal rats, whose scores and pattern of responses were remarkably similar to those of other studies (40,42), the brain-damaged rats displayed no evidence of learning.

Compared to the rats in Stokes and Best's (41) study, where lesions were limited experimentally to the dorsomedial thalamus, the seizure-induced rats' errors were even greater. That the increased severity of deficit is coupled to the nature of the multifocal brain damage in these rats is likely, although we cannot exclude the possibility of circumstantial covert seizures at the time of testing. These results suggest that lesions within different but specific structures, such as the caudate, hippocampus, frontal cortex, and dorsomedial thalamus, may contribute differentially to the reported deficits in RAM performance. Multiple regression analyses (unpublished data) of RAM errors indicate a graduated contribution of these structures to the behavior; however, canonical correlation for both working and reference memory as a function of multifocal damage would be the optimal technique to answer this question.

The behavior of the rats in which seizure-induced brain damage was documented supports Stokes and Best's (41,42) explanation for the deficits in both reference and working memory. They argue that decreased ability to inhibit, stereotyped responses, attentional fluctuations, and deficits in cue utilization (42) determine the impairment in maze performance; memory disruption is considered secondary. Our rats appeared to display all of the correlative behaviors reported for rats in which more than 80% of the dorsomedial thalamus was damaged by experimental lesions; these behaviors include stereotyped responding (13), rapid (disinhibited) responses (e.g., closing jaws on the food dish with sufficient intensity to be heard on the other side of the maze), and increased open field activity (Ross and Therrien, unpublished data). Although we did not correlate the numbers of errors with the measures of brain damage in the present studies: the remarkably consistent pattern of thalamic damage between our experiments and the marked similarity in total errors displayed by rats in this study and the experiment in which quantitative associations were made (14), suggests that the two studies are comparable.

Damage to the dorsomedial thalamus and the suspected mechanisms (such as motor disinhibition and deficits in cue utilization) for the impairment of maze behavior are not necessarily valid for other types of lesions. Knowlton, Shapiro, and Olton (19) have shown that hippocampal seizures disrupt working memory performance but not reference memory acquisition. Kostakos and Persinger (unpublished results) found extremely similar results in adult rats in which lithium-pilocarpine seizures had been induced before weaning; however, these rats did not display the qualitatively gross changes in different domains of behaviors that are reported in the present study.

The second major conclusion of this study is that adult, lithium/pilocarpine-induced brain damage eliminates CTA, relative to normal rats; the effect was less strong in rats that had received 2 mEq/kg of lithium rather than 3 mEq/kg (suggestive of dose dependence) and similar in magnitude to values reported by Venugopal and Persinger (45). Particularly revealing, however, was the propensity for the brain-damaged rats to display less neophobia than normal rats. The rats in which seizures had been induced drank significantly more milk than controls although the treatment groups did not differ during their first consumption of 10% sucrose.

Specific lesions within single structures that comprise the ascending pathways between the solitary nucleus, parabrachial nucleus, hypothalamus (25,44,47), gustatory thalamus (21), amygdala-hippocampal complex (1,5,17), globus pallidus (7), and insular cortices (3,22,23–26) have been shown to disrupt or to attenuate CTA. Our results support Aggleton, Petrides, and Iversen's contention (1) that damage to the basolateral amygdala disrupts appreciation of novel tastes, i.e., reduces normal neophobia and extends the hypothesis of Lasiter et al. (22,23) that anterior insular lesions impair the learning of normal taste aversion. Further quantitative comparisons of multifocal damage within the parabrachial-insular pathways with quantitative measures of the subcomponents of the CTA response might resolve the minor contradictions (27) that have been reported.

One result emerged in our studies that may be applicable to the complex problem of psychogenic seizures. Whereas the occurrence of the spontaneous seizures (months after the induction) increased during the anticipation of food or food-related testing, they decreased in situations that encouraged habituation; these seizures were extremely similar to spontaneous kindled displays (35). Relatively quick elevations in corticosterone and ACTH have been measured in rats that have been maintained (i.e., repeated trials) on food deprivation (18). In light of the essential role of ACTH (and antecedent CRF release via amygdaloid stimulation of hypothalamic neurons) in the display of kindled seizures (38), the possibility that the biochemical correlates (8) of anticipation might trigger spontaneous seizures could be investigated. Because the acceleration in cumulative numbers of spontaneous seizures required several days to develop, the possibility of conditioned elevations in corticosterone that would be associated with the fixed-time food presentations cannot be excluded as a contributing stimulus.

Falter, Persinger, and Chretien (10) found that ACTH levels increased more than tenfold (saturating the assay detection) within 1 h of seizure induction by lithium and pilocarpine. One could argue that subsequent (but less intense) elevations in ACTH could serve as conditioned stimuli. Alternatively, the elevation of ACTH could facilitate state-dependent memory behaviors of which the seizure is one manifestation. That the seizured rats can display state dependence was suggested in this study by the deterioration of reference (long-term) memory

relative to working (short-term) memory when they were administered an anticonvulsant drug after the RAM had been acquired.

The metaphor that the brain is a space-time matrix in which aggregates of elements (neuronal groups) contribute differentially to multiple domains of behavior requires a slight shift in methodological tradition. Subsequent studies will require: quantitative measurements of several different tasks for each rat, careful historical records of each rat's behavior, and the implementation of multivariate statistical analyses, such as canonical correlation, in order to discern the differential contribution of the quantitative continuum of cell loss to domains of behavior that share variance (and presumably similar mechanisms). The results of this approach should both compliment and extend our present understanding of brain–behavior correlates.

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